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Buffer May Be the Critical Factor in Measurement of Anti-Prothrombin Antibody on a γ-Ray-Irradiated Plate by Enzyme-Linked Immunosorbent Assay

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We investigated the influence of different buffers (Tris-buffer and phosphate buffered saline (PBS)/Tween-20 buffer) on anti-prothrombin antibody (aPT) measurement by enzyme-linked immunosorbent assay (ELISA), employing a γ-ray-irradiated plate. We found considerable discrepancies in aPT positivity between each buffer, and we suggest that the use of Tris-buffer is not suitable for aPT measurement with a γ-ray-irradiated plate to measure aPT. © 1996 Wiley-Liss, Inc.

Key words: lupus anticoagulant, anti-cardiolipin antibody, anti-prothrombin antibody, γ-ray-irradiated plate

INTRODUCTION

Recently, Arvieux et al. [1] examined anti-prothrombin antibody (aPT) in patients with lupus anticoagulant (LA) by enzyme-linked immunosorbent assay (ELISA) employing a γ-ray-irradiated plate [2], and they found aPT in 55% of cases. We also confirmed the existence of aPT in 40% of systemic lupus erythematosus (SLE) patients with LA by ELISA, employing a non-γ-ray-irradiated plain plate, but we failed to detect aPT when we employed a γ-ray-irradiated plate [2,3].

The reason for this discrepancy is unclear at this stage, but we hypothesized that differences in the buffers used in these studies may have been a cause. This prediction prompted us to conduct this study.

PATIENTS AND METHODS

Patients

We investigated 15 patients with SLE, including 10 patients with LA or LA/anti-cardiolipin antibody (aCL) and 5 patients without anti-phospholipid antibody (aPA). Positivity of LA was confirmed by the method of Exner et al. [4], and the presence of aCL was confirmed by a method previously reported elsewhere [5]. Thirteen volunteers served as healthy controls.

Measurement of aPT

A plain plate (Dynatech Labs, Inc., Chantilly, VA) or a γ-ray-irradiated plate (10-, 25-, 50- or 100-gray) was coated with 100 μl of purified prothrombin (PT) (25 μg/ml) (Enzyme, South Bend, IN), and washed with Tris-buffered saline (TBS) (0.05 M Tris HCl, 0.15 M NaCL, pH 7.5) [3] or phosphate-buffered saline, pH 7.4, containing 0.1% Tween-20 (PBS/Tween) [1]. Each plate was further coated with fatty acid-free bovine serum albumin (BSA) (Sigma, St. Louis, MO), and washed with TBS or PBS/Tween, and 50 μl of sample serum (×100) was added to each well. After incubation for 1.5 hr and washing with TBS or PBS/Tween, 50 μl of alkaline phosphate-conjugated anti-human IgG (Fc) antibody (×1,000) (Tago, Burlingame, CA) were added, and incubation proceeded for 1 hr. Color was developed by adding p-nitrophenol phosphate/NaOH and measured at 405 nm. Each experiment was carried out in duplicate, and aPT was judged positive when the optical density (OD) of the sample exceeded the mean value (+2 SD) of the healthy controls. We also added phosphatidyl serine (PS) and/or

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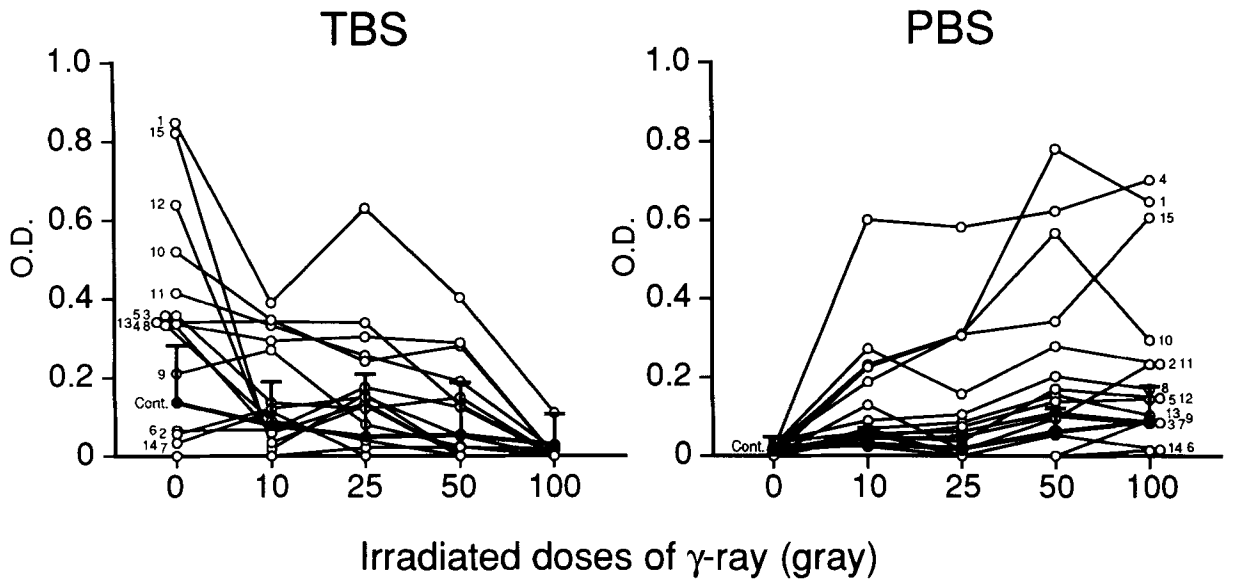

Fig. 1. Effects of Tris-buffered saline and phosphate-buffered saline/Tween-20 on positivity of antiprothrombin antibody by enzyme-linked immunosorbent assay (ELISA) employing plain- and/or γ-ray-irradiated plate. TBS, Tris-buffered saline; PBS, phosphate-buffered saline containing 0.1% Tween-20. Nos. 1–15, patients.

Ca^{2+} to each well together with PT to examine the influence of PS and/or Ca^{2+} on aPT measurement. The specificity of aPT was confirmed by an absorption experiment using a method previously reported elsewhere [3].

RESULTS

aPT was positive in 10 SLE patients with LA or LA/aCL, and negative in the remaining 5 patients without aPL using a plain plate and TBS. When a γ-ray-irradiated plate was employed, the OD readings of each aPT-positive sample confirmed by using a plain plate gradually decreased, showing reverse correlation with the irradiated doses of γ-ray (Fig. 1). Positivity of aPT in 10-, 25-, 50-, and 100-gray irradiated plates was 50%, 40%, 20%, and 0%, respectively. None of the samples measured with PS and/or Ca^{2+} was positive for aPT, thus showing the lack of influence of PS/Ca^{2+} on aPT measurement of whole serum using a γ-ray irradiated plate and TBS.

On the other hand, aPT was negative in all 15 patients with SLE when using plain plate/PBS. aPT was positive in 6, 7, 8, and 6 of 10 SLE patients (all positive for aPT in a plain plate and TBS) using 10-, 25-, 50-, and 100-gray irradiated plates and PBS, respectively. Thus, 2 of 10 SLE patients judged to be positive for aPT by plain plate and TBS were judged negative for aPT by a γ-ray-irradiated plate and PBS, and one SLE patient judged negative for aPT by a plain plate and TBS was found to be positive for aPT by a plate and PBS. However, this was revealed to be a false-negative reaction for aPT by the absorption experiment. Addition of Ca^{2+} and/or PS caused marked decreases in the OD readings, thereby leading to false-negative reactions in all of the γ-ray-irradiated plates.

DISCUSSION

We confirmed a marked decrease in positivity of aPT when using a γ-ray-irradiated plate and TBS compared to the level when we employed a plain plate and TBS. On the contrary, aPT positivity was increased with the use of a γ-ray-irradiated plate and PBS compared to the level obtained with a plain plate and PBS, thus suggesting the possibility that a cause of the discrepant results obtained by Arvieux et al. [1] and our earlier study [3] is the difference in the buffers employed in each investigation.

It is possible that the ingredient(s) of TBS may react to and block a radical(s) introduced on the surface of the plate by γ-ray irradiation, which is indispensable for modification of PT as an antigen as in the case of β_2 GPI [2,6,7]. However, any explanation remains speculative.

These results indicate that the type of plate and buffer are important factors in the measurement of aPT, and that standardization of aPT measurement may be required in the near future.

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